

### ***Transfection (calcium phosphate-based protocol)***

1. seed cells on 6 well plates
2. 24 h post seeding, control cell confluent (optimal around 50%- 70%)
3. Bring  $\text{CaCl}_2$  (2.5 M) to room temperature and pre-warmed HPSS at 37°C
4. In 50 ml falcon tube, add plasmids (12.5  $\mu\text{g}$  from each), 250  $\mu\text{l}$  of  $\text{CaCl}_2$ , water (sterile- open only in hood) up to 1 ml, and mix gently
5. Add 1 ml HPSS- drip slowly!
6. Vortex the falcon. The liquid will become cloudy
7. Add 200  $\mu\text{l}$  (1.25  $\mu\text{g}$  plasmid) from the transfection solution dropwise to selected wells in the culture plate- Mix gently
8. Place plates back to the incubator